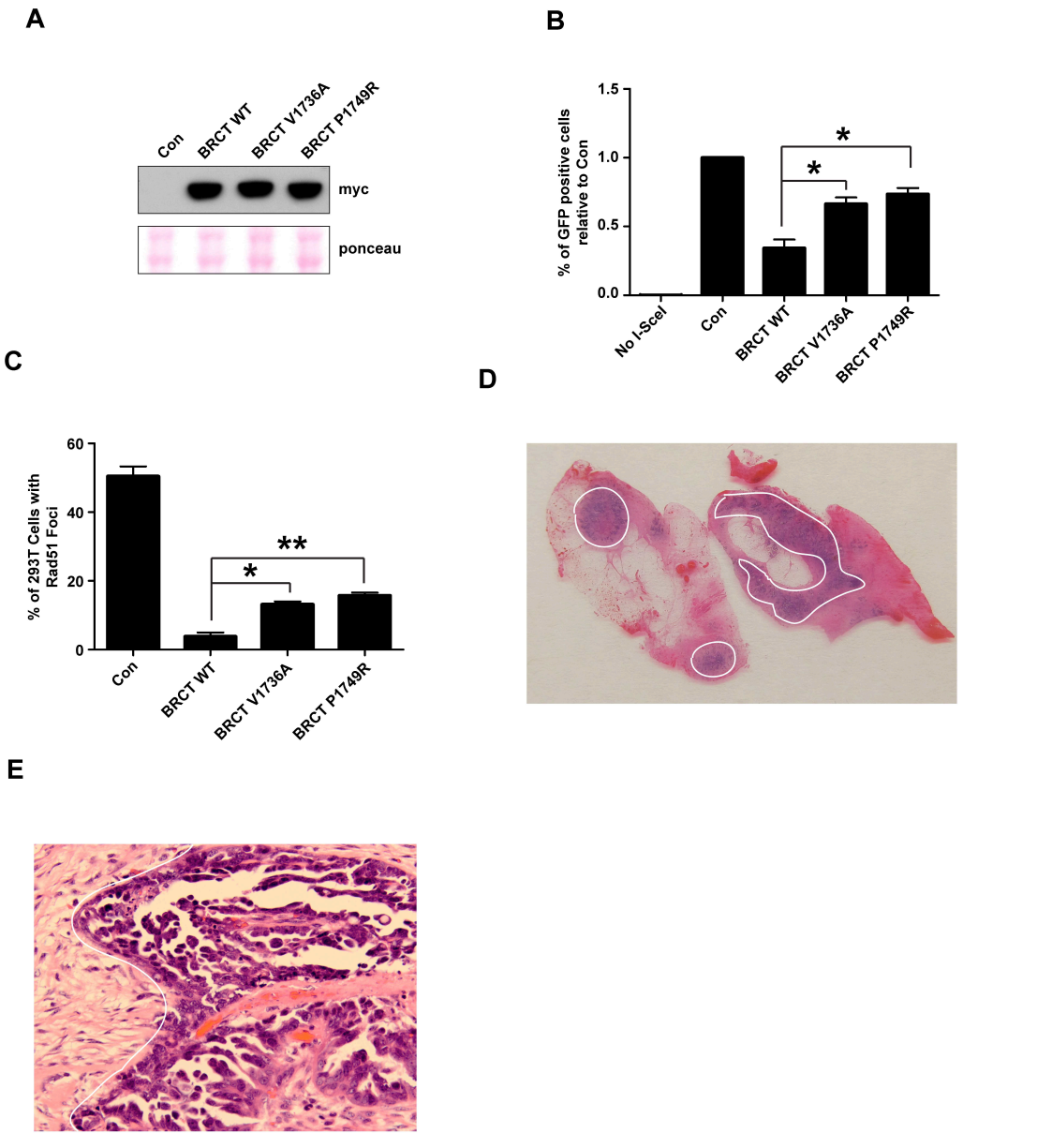
Supplementary Figure 3



Supplementary Figure 3. Analysis of V1736A in DNA repair and preparation of genomic DNA samples from tumor samples.

A, similar ectopic expression levels of Myc-BRCT WT, V1736A and P1749R in transfected cells. 293T cell were transfected with 1ug of Myc-BRCT WT, V1736A or P1749R. Whole cell extracts were analyzed by Immunoblot with an anti-Myc antibody (9E10). Ponceau staining was used to show equal loading.

B, Expression of BRCA1 BRCT impaired homology directed repair of I-SceI-induced DSBs as assessed in a DR-GFP reporter system. U2OS DR-GFP cells were co-transfected with expression vector of I-SceI and Myc-BRCT WT, V1736A or P1749R. 3 days later, percentage of GFP positive cells were determined by FACs as a read out of efficiency of homology-direct repair of DSBs. Error bars represent S.E.M of 3 experiments and * $p< 0.05$.

C, Expression of BRCA1 BRCT decreased accumulation of Rad51 at DSBs. 293T cells were transfected with Myc-BRCT WT, V1736A, P1749R or a control vector. 24 hours later, cells were irradiated (10 Gy) and incubated for 6 hours before fixation and staining for IF. Cells were stained for both Rad51 (H92) and Myc (9E10). For control transfected cells, percentage of cells with Rad51 foci was counted. For Myc-BRCT transfected cells, percentage of Myc positive staining cells with Rad51 foci was counted. Error bars represent S.E.M of 3 experiments and * $p<0.05$, ** $p< 0.01$.

D, Selection of ovarian cancer tissue for genotyping. An HE stained slide with ovarian cancerous tissues circled in white lines is shown. This slide is used to mark the positions of cancerous tissue. Using unstained slides, tissues outside of the circles were first removed and those inside the circled were collected for DNA extraction and genotyping.

E, A microscopic picture of the HE stained slide as in D is shown. A white line is used to separate the stroma and cancerous tissue.